



REVIEW

DNA electrotransfer: its principles and an updated review of its therapeutic applications

F André and LM Mir

Laboratory of Vectorology and Gene Transfer, UMR 8121 CNRS – Institut Gustave-Roussy, Villejuif Cedex, France

The use of electric pulses to transfect all types of cells is well known and regularly used *in vitro* for bacteria and eukaryotic cells transformation. Electric pulses can also be delivered *in vivo* either transcutaneously or with electrodes in direct contact with the tissues. After injection of naked DNA in a tissue, appropriate local electric pulses can result in a very high expression of the transferred genes. This manuscript describes the evolution in the concepts and the various

optimization steps that have led to the use of combinations of pulses that fit with the known roles of the electric pulses in DNA electrotransfer, namely cell electroporation and DNA electrophoresis. A summary of the main applications published until now is also reported, restricted to the *in vivo* preclinical trials using therapeutic genes.

Gene Therapy (2004) 11, S33–S42. doi:10.1038/sj.gt.3302367

Keywords: electrogenotherapy; DNA electrotransfer; nonviral gene therapy; electroporation; electroporation; electroporation; naked DNA

Introduction

Nonviral gene therapy using the combination of 'physical' approaches and naked DNA is rapidly developing for two main reasons: the use of naked DNA eliminates the limitations and the risks linked to the use of viruses (coding sequence length in the case of the adenovirus associated virus, insertional mutagenesis in the case of the retrovirus, immunological responses in the case of the adenoviruses, etc) and, in spite of extensive research, efficient and safe chemical vectors have not yet been developed for *in vivo* gene delivery.

There are several physical approaches for nonviral gene therapy. (i) The simplest, of course, is the injection of naked DNA, which in the skeletal or cardiac muscle leads to some expression of the injected genes;^{1–3} however, this expression is very low and very variable from sample to sample. (ii) The hydrodynamic method consists in the very rapid injection through the mouse tail vein of a large volume of DNA solution: it results in a very efficient transfection of liver cells, even though the procedure is somehow dangerous for the treated mice;^{4–6} indeed, part of the mechanism is based on the transient heart failure resulting from the injection, which blocks the fluid distribution in the body and provokes a liquid overpressure in the liver.⁶ (iii) For physical DNA transfer to superficial tissues like the skin or the leaves in the plant kingdom, the 'biolistic' approach ('jet injection' and 'gene gun') also leads to good transfection levels. The commonly termed 'gene gun' consists in a device propelling plasmid-coated gold microparticles.^{7,8} For the 'jet injection' or 'needle-free' injection, the DNA is pushed at high pressure and high speed through a tiny

orifice at the head of the injector, creating an ultrafine stream of high-pressure fluid that penetrates the skin.^{9,10} (iv) The proof of the concept of sonoporation (use of focused ultrasounds to permeate the cells) has just been developed and still requires further elaboration.¹¹ (v) DNA electrotransfer has been used with success since 1998 and is becoming a real alternative to the viral methods for *in vivo* gene transfer.

The use of electric pulses is very popular for the transfection of bacterial and eukaryotic cells *in vitro*. The initial limitation of the so-called electroporation, a low cell survival, could be overcome by the use of appropriate electric pulses. The technique was then transferred *in vivo*, and termed DNA electrotransfer or electrogenotherapy. In this article, we present an historical survey of this approach, which will include the description of the bases of cell electroporation and DNA electrotransfer, as well as several consecutive optimization efforts that have led to a very efficient and safe procedure. A summary of the main applications published until now is also reported, restricted to the *in vivo* preclinical trials using therapeutic genes.

The origin of DNA electrotransfer (the *in vitro* only period)

The first pioneering demonstration that DNA could be introduced into living cells by means of electric pulses was published by E Neumann in 1982.¹² He built a device with chambers specifically designed for the pulse delivery to the suspension of cells and DNA. More than 2 years were necessary before the publication of the second paper describing successful transfer of DNA to eukaryotic cells *in vitro* by H Potter in 1984.¹³ Since this result

was achieved using a classical (thus accessible) laboratory equipment, the ISCO 494 generator for proteins and DNA gel electrophoresis, many other groups could try this approach. The procedure consisted in creating a short circuit through the cell suspension, which caused the delivery of an exponentially decaying electric pulse to the cells. Since then, devices delivering exponentially decaying pulses have been developed by various companies.¹⁴ However, already in 1985, J. Teissié developed the first square wave pulse generator with outputs compatible with the needs for cell electroporation in *vitro*.¹⁵ In any case, since 1986, DNA electrotransfer is the most popular way to transfect bacterial cells and one of the good options for the *in vitro* transfection of eukaryotic cells as well.

Principles of the DNA electrotransfer

The exposure of living cells to short and intense electric pulses induces position-dependent changes in the transmembrane potential difference. These changes are well described by the equation of Schwann, which indicates that the value of the induced change is proportional to the cell radius and the scalar value of the external electric field (Figure 1). This change will superimpose to the resting transmembrane potential. When the transmembrane potential difference net value (the sum of the vectorial values of the induced and resting potential differences) is greater than 0.2–0.4 V, transient permeation structures are generated at the cell membrane level, because the membrane structure cannot resist the electrocompressive forces due to this potential difference. Electroporation is thus a threshold phenomenon, imposed by the need to overpass a threshold value of the transmembrane potential difference.

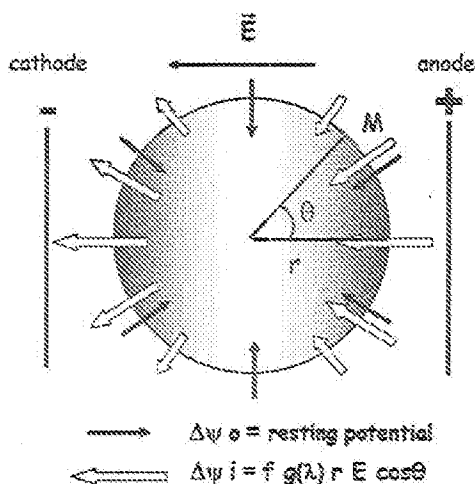


Figure 1 Effect of an external electric field applied on a living cell. The external electric field induces a change ($\Delta\psi_i$) in the resting transmembrane potential ($\Delta\psi_o$) of the cell. The value of the induced change depends on the shape of the cell (f) and the conductivity of the media $g(\lambda)$. At point M on the cell surface, it is also proportional to the cell radius (r), the scalar value of the external electric field (E) and the cosine of the angle θ (polar coordinate of point M).

The structure of these transient permeation structures is not yet elucidated. Some models proposed the generation of 'electropores' (described as 'holes' in the membranes), but cell electroporation can be totally reversible and the theory hardly explains the resealing of these 'electropores'. Recent modelization by molecular dynamics¹⁶ has suggested that under transmembrane potential differences, much larger than those necessary to obtain the 'physiological' reversible cell electroporation, pores could indeed be generated. Still, the reversible structure remains undefined.

Properties of cell electroporation: cell membrane crossing

Cell electroporation is a general phenomenon that can be obtained in the cells of archaeobacteria, eubacteria and eucarya phyla. Indeed, all living cells are limited by a nonconductive membrane that isolates the internal conductive medium from the external medium. Thus, all cells will react to external electric fields by the induction of a transmembrane potential difference that, above a threshold value, will provoke the membrane destabilization. Under appropriate electrical parameters, this destabilization will be totally reversible, ensuring the survival of the transiently permeabilized cell. Owing to the use of very short pulses, cell electroporation is a nonthermal phenomenon: this characteristic contributes to its reversibility (no denaturation of the membrane proteins, even though one of the cell electroporation early models, which was not validated, considered protein denaturation as the *primum movens* of the membrane properties changes).¹⁷ Finally, the most interesting property of the cell electroporation, which supports several biomedical and biotechnological applications, is the fact that cell electroporation allows the direct delivery of nonpermeant molecules inside the cell cytoplasm, bypassing the normal internalization route for these molecules (usually the endocytosis pathway). Small nonpermeant molecules can enter the electroporated cells by diffusion through the transiently permeabilized cell membrane, while large nonpermeant molecules like DNA enter by other mechanisms as discussed below.

In vivo delivery of electric pulses

Before the *in vivo* delivery of electric pulses in the frame of the electrogenotherapy, other applications of the *in vivo* cell electroporation were developed. In 1989 and 1990, Grasso *et al.*¹⁸ Grasso and Heller¹⁹ applied electric pulses *in vivo* to rabbit cornea, in order to fuse human HeLa cells to the cells of the cornea. Cell electrofusion, *in vitro*, is the consequence of the electroporation of two adjacent cells or of the fact that two previously electroporated cells are brought in close contact. In 1988, it was found that bleomycin toxicity is several hundreds of thousands times higher on electroporated cells than on cells unexposed to the electric pulses.²⁰ This increase in toxicity was also found in preclinical experiments in which permeabilizing electric pulses were delivered transcutaneously to

transplanted²¹ or spontaneous tumours in mice.²² Moreover, because of the antitumour efficacy of this approach, clinical trials were rapidly performed.^{23,24} This approach was termed electrochemotherapy, and the pulse conditions, used in almost all the published clinical trials,²⁵⁻²⁷ were eight identical square wave pulses of 100 μ s and 1300 V/cm at a repetition frequency of 1 Hz using external electrodes (transcutaneous pulses). These trials demonstrated that it is possible to deliver *in vivo* electric pulses to animals and patients and they greatly facilitated the development of the *in vivo* DNA electrotransfer.

Indeed, in parallel, Jon Wolff showed in 1990¹ that direct injection of naked DNA in skeletal muscle *in vivo* results in gene expression at low and variable levels. Thus, it seemed tempting to combine the injection of DNA and the electric pulses delivery.

In 1991, Titomirov *et al.*²⁸ delivered exponentially decaying short pulses to skin after myc and ras genes injection and they were able to recover a few growing cells expressing myc and ras proteins that could, eventually, reflect the *in vivo* electrotransfer of these oncogenes. In 1996, Heller *et al.*²⁹ delivered electrochemotherapy-type trains of short pulses (100 μ s) to the liver after the injection of reporter genes DNA, with good levels of transfection that nowadays could also be partly explained by the injection itself, taking into account the results of the hydrodynamics method. In 1998, four groups, in three different tissues, consistently demonstrated good transfection levels using long pulses (5–50 ms): MP Rols and J Teissié in tumours,³⁰ Suzuki *et al.* in the liver,³¹ and Aihara and Miyazaki,³² and Mir *et al.*³³ in the skeletal muscle. The use of trains of several identical pulses in the milliseconds to tens of milliseconds duration range actually results in a highly significant increase in the level of expression of the reporter genes coded by the naked DNA injected in the target tissues. Later on, Lucas and Heller³⁴ compared short and long pulses, demonstrating that the level of expression was higher, and expression duration longer, when long pulses were delivered into the tissues.

Use of trains of identical electric pulses for efficient DNA electrotransfer in skeletal muscle

The earliest and most exhaustive series of experiments allowing to understand the mechanisms of DNA electrotransfer as well as to optimize such trains of identical electric pulses were reported in 1999.³⁵ Experiments analysed the respective influence of the pulse duration, voltage applied (or more precisely of the applied voltage to electrodes distance ratio), number of pulses and repetition frequency. Using the gene coding for the firefly luciferase, an increase of 200 times of the expression with respect to the naked DNA injection alone was shown, a large decrease in the variability of this expression, as well as a long-term expression since the high level of expression remained stable for at least 9 months. In the mouse skeletal muscle, using external electrodes (transcutaneous electric pulses) and trains of identical electric pulses, optimal conditions are eight pulses of 20 ms and 200 V/cm at a repetition frequency

of 1 or 2 Hz, delivered after the intramuscular injection of the DNA).^{36,37} These conditions have been adapted to other tissues: in tumours, transfection levels that depended on the tumour type were found maximal using eight identical pulses of 20 ms and 500 or 600 V/cm at a repetition frequency of 1 or 2 Hz,³⁸ 250 V/cm in the liver,³⁹ and 500 or 750 V/cm for the skeletal muscle in neonate mice (7–10 days old mice).⁴⁰

Roles of the electric pulses in DNA electrotransfer

Experiments showed that, after the electric pulses delivery, tissues remain permeabilized for several minutes.^{35,41,42} Moreover, for an efficient transfer, DNA must be injected before the electric pulses delivery. Thus, permeabilization of the cells is not sufficient even though it is necessary since efficient electrotransfer requires sufficiently intense electric fields (above cell permeabilization threshold).^{36,37} Moreover, efficient electrotransfer requires sufficiently long pulses. The mechanism of DNA electrotransfer could not be just cell electropermeabilization and DNA diffusion through the permeabilized plasma membrane.

The role of the electric pulses in DNA electrotransfer has then been studied using combinations of pulses. Instead of delivering trains of eight identical pulses (of 20 ms and 200 V/cm, at a repetition frequency of 1 Hz), cells were exposed to:

- 1 HV (high voltage, short pulse) of 100 μ s at 800 V/cm (an electrochemotherapy-like pulse, with a field strength adapted to the skeletal muscle, the muscle fibres having a diameter larger than the average diameter of the tumour cells; using eight of such pulses at 1 Hz repetition frequency, Gehl *et al.*³⁷ showed that this field strength was the highest that one could deliver to the muscle fibres without provoking their irreversible electropermeabilization).
- followed by
- 1 or several LV (low voltage, long pulse) of 100 ms at 83 V/cm (nonpermeabilizing pulses, of a field intensity below the threshold for reversible permeabilization in the mice skeletal muscle).⁴³

The first experiments were performed using two classical square wave generators, with LV of 83 ms, a limitation imposed by the devices used, and a manual switch to deliver the LV(s) after the HV.⁴³ Then a device for the controlled generation of such combinations of HV and LV pulses, with, moreover, a controlled gap between the HV and LV pulses, was prepared by the Faculty of Electrical Engineering of the University of Ljubljana, based on a previously developed electroporator.⁴⁴ With such an equipment, the roles of the electric pulses in DNA electrotransfer could actually be analysed.

The efficacy of several combinations of pulses (1 HV alone, or 1 HV followed by 1 LV, or 1 HV followed by 4 LV) was compared.⁴² It was shown that the duration of the high permeabilized state of the muscle fibres was the same for the three combinations tested, all of them including the same HV pulse. On the contrary, the authors found that efficacy was only achieved if at least

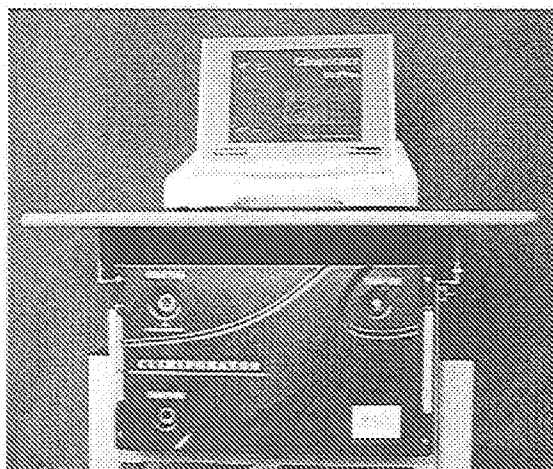


Figure 2 The Cliniporator. This new pulse generator (Cliniporator™, IGEA s.r.l., Carpi, Italy) has been developed within the Cliniporator project (QLK3-1999-00484) of the 5th Framework Program of the European Union. It delivers combinations of HV and LV pulses.

an LV was delivered after the HV. They also reported that the efficacy of a single LV could be observed even if this LV was delivered up to 100 s after the HV, while in the case of the delivery of 4 LV, efficacy could be achieved even if the 4 LV were delivered up to 3000 s after the HV. Since almost no efficacy was found with the LV alone, the conclusions of the authors were that the HV pulses delivery (permeabilizing pulses) were mandatory, but that the efficacy of the procedure was brought by the LV pulses. Other arguments have contributed to point out that the long LV pulses act on DNA, provoking its electrophoretic displacement.⁴⁵

The electric pulses thus have two roles, the 'electroporation' of the target cells and the electrophoretic transport of the DNA 'towards or across' the cell membrane. Target cell electroporation is mandatory, but the electrophoretic component of the electric pulses is actually instrumental in DNA electrotransfer efficacy.

These combinations have been studied within the Cliniporator project (QLK3-1999-00484) of the 5th Framework Program of the European Union. Moreover, a new pulse generator (Cliniporator™, IGEA s.r.l., Carpi, Italy), able to deliver these combinations of pulses, has been developed within this project (Figure 2). In the skeletal muscle and the skin, appropriate pulses parameters have led to a further increase of the expression of the luciferase coding plasmid with almost no histological modification.

Interests of DNA electrotransfer

In vitro, DNA electrotransfer is interesting because it is based on cell electroporation, which is the perturbation of cell membrane impermeability by physical means, with neither addition nor withdrawal of membrane components as it happens when chemical permeabilization means are used. Thus, full recovery is facilitated. Moreover, if actually controlled, cell electroporation is a nonthermal effect, without protein

denaturation (which also facilitates cell recovery). Moreover, the method is simple, since it only requires to mix the cells and the DNA and to pulse the mixture.

In vivo, DNA electrotransfer is also interesting because it allows the transfer of genes into tissues without using virus. Moreover, no chemical method works *in vivo* better than the direct electrotransfer of the naked DNA. The method is also very rapid: the new constructs made by usual molecular biology approaches can be amplified by rapid 'minipreparations' of DNA and quantified by optical density determination; then it is sufficient to adjust plasmid concentration, to inject, and to 'pulse' (with viral methods, constructs must be inserted in a viral background, transfected in producing cells, and then virions must be produced, collected, isolated, concentrated and titrated before they could be injected).

Therapeutic applications already developed in preclinical trials

Tables 1 and 2 summarize the result of an extensive search for publications reporting the *in vivo* delivery of genes of therapeutic interest by means of DNA electrotransfer. Publications using only reporter genes have not been included in the tables. Experiments have been classified according to the main applications foreseen by their authors.

Most of the experiments deal with gene transfer to the skeletal muscle in mice. However, gene transfer to tumours, brain, lumbar intrathecal space, skin, liver, cornea, brain, penile corpora cavernosa and seminiferous tubes have also been reported. Experiments, mainly in mice (about 80% of the publications) have also been performed in rats, pigs, rabbits, guinea pigs, sheep, goats, dogs and cattle. These experiments also demonstrate the safety of the procedure, the possibility to repeat the treatment (shown already in 1999 by Rizzutto *et al.*¹²⁰), as well as the possibility to coelectrotransfer up to three plasmids into the same skeletal muscle fibres.⁸⁸ The main general application is immunotherapy (48%; 54/113). Cancer treatment (38%; 43/113), metabolic disorders or metabolism modification (17%; 19/113) and correction of organ or site-specific diseases (14%; 16/113) are the three other frequent applications. Monogenetic diseases (9%; 10/113), cardiovascular diseases (9%; 10/113) and analgesia (2%; 2/113) are other applications also found in the literature.

It must be noted that each of these applications includes the use of a large variety of genes. In this respect, it is necessary to point out that the genes of proteins involved in the immune system responses have been the most usually transferred genes for vaccination, cancer, treatment of arthritis, immunological protocols, etc. These genes include those coding for the IL-2, IL-4, IL-10, IL-12, IL-18, IL-18 binding protein, soluble TNF receptor, GM-CSF, tumour epitopes, the HIV gag gene, recombinant monoclonal antibodies, mycobacterial antigens, etc. The details are listed in the Table 2. In fact, this observation must be related to the fact that in many cases the transfected tissue is the skeletal muscle, used as a cell factory for the production of factors that will act systemically on distant targets.

Therapeutic levels have been achieved. For example, in the case of the gene coding for the erythropoietin

Table 1 Applications of the *in vivo* delivery of genes of therapeutic interest by means of DNA electrotransfer

Applications*	Diseases*	Genes	Tissues	Animals	Ref.
Analgesia 2 Cancer 43	Analgesia 2	PGMC (proopiomelanocortin)	Intrathecal space	Rat	46, 47
	Cancer 43	IL-2; IL-12; IL-18; INF α ; GM-CSF; CpG containing DNA; full TRP-2 or epitopes; diptheria toxin; HSV TK; TIMP; p53; bcl-2; MBD-2; antisense VEGF; Flk-1 VEGF receptor; metargidine (MDC-15); Stat3 variant; K1-5; K1-3-HA5; endostatin	Muscle, tumour, skin, liver	Mouse, rat	34, 48-87, 101, 102
Cardiovascular diseases 10	Atherosclerosis 2	IL-12; human plasma platelet-activating factor acetylhydrolase (PAF-AH)	Muscle	Mouse	88, 89
	Ischaemia 4	IL-10; IL-18; NF- κ B binding sites containing DNA; hVEGF-A and hVEGF-B; protein-disulfide isomerase	Muscle, right hippocampus	Mouse, rat	90-93
Immuno-therapy 54 Metabolic disorders 19	Myocarditis 4	IL-1 α ; IL-10	Muscle	Mouse, rat	94-97
	See Table 2	See Table 2	See Table 2	See Table 2	
	Anaemia 10	Epo; dimeric erythropoietin fusion protein	Muscle, skin	Mouse, rat	113-122
	Diabetes 6	IL-4; insulin precursors; codelivery of B7-1 and PPIs or CEA; IGF-1	Muscle	Mouse	123-128
Monogenetic diseases 10	Neuropathy 1	NT3 neurotrophin3	Muscle	Mouse	129
	Thrombocytopenia 2	Recombinant human thrombopoietin (ritipo)	Muscle	Mouse	130, 131
	β -Thalassemia 2	Epo	Muscle	Mouse	132, 133
	Haemophilia B 1	Factor IX	Muscle	Mouse, dog	134
Organ- or tissue-specific diseases 16	Mucopolysaccharidosis 1	IDS (iduronate-2-sulphatase)	Muscle	Mouse	135
	Myodystrophy 5	Dystrophin or minidystrophin; laminin α 2; CA-binding protein	Muscle	Mouse	136-140
	Neuron degeneration 1	Cardiostrophin	Muscle	Mouse	40
	Arthritis 6	IL-10; PCMC (proopiomelanocortin); soluble TNF Receptor	Muscle	Mouse, rat	95, 141-145
Organ- or tissue-specific diseases 16	Bone formation 1	BMP-4	Muscle	Mouse	146
	Bronchopulmonary hyperactivity 1	IL-10	Muscle	Mouse	112
	Erectile dysfunction 1	Neuronal NOS (nitric oxide synthase), penile NOS	Penile corpora cavernosa	Rat	147
	Gastric disorders 1	Gastrin	Muscle	Mouse, rat	148
Organ- or tissue-specific diseases 16	Kidney regeneration 1	HGF	Muscle	Rat	149
	Liver regeneration 2	HGF	Muscle	Mouse, rat	150, 151
	Muscle regeneration 2	IGF-1	Muscle	Mouse	126, 152
	Ocular diseases 1	Human tPA (tissue plasminogen activator)	Corneal endothelium	Rat	153
Organ- or tissue-specific diseases 16	Spermatogenesis rescue 1	Stem cell factor (SCF) cytoplasmic domain	Seminiferous tubules	Mouse	154

*Number in bold letters corresponds to the number of publications concerning the application or disease.

Table 2 Details of the applications involving immune system-related genes (cytokines, antigens, etc)

Applications ^a	Diseases ^a	Genes	Tissues	Animals	Ref.
Cancer 27	Cancer 27	IL-2; IL-12; IL-18; INF α ; CpG containing DNA; GM-CSF; FLT3-L; R1ak1; TAP-2 or epitopes	Muscle, tumour, skin	Mouse	34, 48-70, 76, 101, 102
Cardiovascular diseases 7	Atherosclerosis 1 Ischaemia 2 Myocarditis 4	IL-12 IL-10; IL-18 IL-13; IL-10	Muscle	Mouse	88
Metabolic disorders 2	Diabetes 2	IL-4; Codelivery of B7-1 or B7-1va	Muscle	Mouse, rat	88, 90 94-97
Organ- or tissue-specific diseases 6	Bronchopulmonary hyperactivity 1 Arthritis 5	IL-4; Codelivery of B7-1 or B7-1va IL-10; soluble p75 TNF receptor linked to the Fc portion of human IgG1 (sTNFR-Fc)	Muscle	Mouse	123, 125 112
Vaccination 12		INF α ; HBsAg; HA influenza virus; HIV gag; Japanese encephalitis virus; mycobacterial antigens; recombinant mAb; chains of the Tg10 mouse mAb; chimeric hepatitis C virus env2 glycoprotein; B10 t3	Muscle, skin	Mouse, pig, sheep, goats, cattle, rabbit, guinea pig	95, 142-145 96-100, 103-111

^aNumbers in bold letters correspond to the number of publications concerning the application or disease.

(epo), hematocrit increase has been achieved in many cases (Table 1). Expression of electrotransferred mini-dystrophin gene in the altered myodystrophic muscles of the mdx mice has been demonstrated (Table 1). The electrotransfer of the genes coding for antioangiogenic factors has demonstrated distant antitumour effects as well as antimetastatic effects in the murine model consisting in the intravenous injection of B16F10 cells.^{80,82} Noticeable concentrations of the cytokines Interleukin-2 and GM-CSF in tumours transfected with the corresponding genes have been measured.⁸⁹ The biological effects observed in the publications listed in Table 1 demonstrate the efficacy of the electrogenotherapy for the treatment of various diseases.

Finally, DNA electrotransfer has also been used for biotechnological purposes. For example, the electrotransfer of the human erythropoietin gene in the oviduct of laying hens has also been carried out for the production of the human erythropoietin,¹⁵⁹ and the transfer of the growth hormone-releasing hormone has also been successfully performed in pigs, not for the treatment of a pig disease but for the achievement of an enhanced weight gain and improved body composition.¹⁵⁶⁻¹⁵⁸

Conclusion

In conclusion, DNA electrotransfer or electrogenotherapy constitutes a real alternative to viral approaches for gene transfer *in vivo*. Its efficacy is proven and there is no doubt on its biological safety. Moreover, DNA preparation is easy and secure, the roles of the electric pulses are described, the control of transfer conditions is achievable and appropriate equipment is available.

Acknowledgements

We acknowledge the financial support of CNRS, IGR, AFM and the EU Commission through the projects Cliniporator (QLK3-1999-00484)¹⁵⁹ and Esope (QLK3-2002-02003) coordinated by LMM. We also acknowledge all their colleagues for fruitful discussions and collaborative work. FA is the recipient of an 'aide aux études' from the AFM (Association Française contre les Myopathies).

References

- Wolff JA *et al.* Direct gene transfer into mouse muscle *in vivo*. *Science* 1990; 247: 1465-1468.
- Budker V *et al.* Hypothesis: naked plasmid DNA is taken up by cells *in vivo* by a receptor-mediated process. *J Gene Med* 2000; 2: 76-88.
- Satkauskas S, Bureau ME, Mahfoudi A, Mir LM. Slow accumulation of plasmid in muscle cells: supporting evidence for a mechanism of DNA uptake by receptor-mediated endocytosis. *Mol Ther* 2001; 4: 317-323.
- Liu F, Song Y, Liu D. Hydrodynamics-based transfection in animals by systemic administration of plasmid DNA. *Gene Therapy* 1999; 6: 1258-1266.
- Liu F, Huang L. Improving plasmid DNA-mediated liver gene transfer by prolonging its retention in the hepatic vasculature. *J Gene Med* 2001; 3: 569-576.

- 6 Zhang G *et al.* Hydroporation as the mechanism of hydrodynamic delivery. *Gene Therapy* 2004; 11: 675-682.
- 7 Udvardi A *et al.* Uptake of exogenous DNA via the skin. *J Mol Med* 1999; 77: 744-750.
- 8 Godon C, Caboche M, Daniel-Vedele F. Transient plant gene expression: a simple and reproducible method based on flowing particle gun. *Biochimie* 1993; 75: 591-595.
- 9 Furth PA, Shamay A, Wall RJ, Hennighausen L. Gene transfer into somatic tissues by jet injection. *Anal Biochem* 1992; 205: 365-368.
- 10 Walther W *et al.* Intratumoral low-volume jet-injection for efficient nonviral gene transfer. *Mol Biotechnol* 2002; 21: 105-115.
- 11 Wang G *et al.* Ultrasound-guided gene transfer to hepatocytes *in utero*. *Fetal Diagn Ther* 1998; 13: 197-205.
- 12 Neumann E, Schaefer-Ridder M, Wang Y, Hofschneider PH. Gene transfer into mouse lymphoma cells by electroporation in high electric fields. *EMBO J* 1982; 1: 841-845.
- 13 Potter H, Weir L, Leder P. Enhancer-dependent expression of human kappa immunoglobulin genes introduced into mouse pre-B lymphocytes by electroporation. *Proc Natl Acad Sci USA* 1984; 81: 7161-7165.
- 14 Puc M *et al.* Techniques of signal generation required for electroporation: Survey of electroporation devices. *Bioelectrochemistry* 2004; 64: 113-124.
- 15 Zerbib D, Amalric F, Teissie J. Electric field mediated transformation: isolation and characterization of a TK+ subclone. *Biochem Biophys Res Commun* 1985; 129: 611-618.
- 16 Tieleman DP, Leontiadou H, Mark AE, Marrink SJ. Simulation of pore formation in lipid bilayers by mechanical stress and electric fields. *J Am Chem Soc* 2003; 125: 6382-6383.
- 17 Tsong TY, Su ZD. Biological effects of electric shock and heat denaturation and oxidation of molecules, membranes, and cellular functions. *Ann NY Acad Sci* 1999; 888: 211-232.
- 18 Grasso RJ, Heller R, Cooley JC, Haller EM. Electroporation of individual animal cells directly to intact corneal epithelial tissue. *Biochim Biophys Acta* 1989; 980: 9-14.
- 19 Heller R, Grasso RJ. Transfer of human membrane surface components by incorporating human cells into intact animal tissue by cell-tissue electroporation *in vivo*. *Biochim Biophys Acta* 1990; 1024: 185-188.
- 20 Orlowski S, Belehradek Jr J, Paoletti C, Mir LM. Transient electroporation of cells in culture. Increase of the cytotoxicity of anticancer drugs. *Biochem Pharmacol* 1988; 37: 4727-4733.
- 21 Mir LM, Orlowski S, Belehradek Jr J, Paoletti C. Electrochemotherapy potentiation of antitumor effect of bleomycin by local electric pulses. *Eur J Cancer* 1991; 27: 68-72.
- 22 Belehradek Jr J *et al.* Electrochemotherapy of spontaneous mammary tumours in mice. *Eur J Cancer* 1991; 27: 73-76.
- 23 Mir LM *et al.* Electrochemotherapy, a new antitumor treatment: first clinical trial. *C R Acad Sci III* 1991; 313: 613-618.
- 24 Belehradek M *et al.* Electrochemotherapy, a new antitumor treatment. First clinical phase I-II trial. *Cancer* 1993; 72: 3694-3700.
- 25 Mir LM *et al.* Effective treatment of cutaneous and subcutaneous malignant tumours by electrochemotherapy. *Br J Cancer* 1998; 77: 2336-2342.
- 26 Cothelf A, Mir LM, Gehl J. Electrochemotherapy: results of cancer treatment using enhanced delivery of bleomycin by electroporation. *Cancer Treat Rev* 2003; 29: 371-387.
- 27 Serra C, Cemazar M, Rudolf Z. Electrochemotherapy: advantages and drawbacks in treatment of cancer patients. *Cancer Ther* 2003; 1: 133-142.
- 28 Tiliomirov AV, Sukharev S, Kistanova E. *In vivo* electroporation and stable transformation of skin cells of newborn mice by plasmid DNA. *Biochim Biophys Acta* 1991; 1088: 131-134.
- 29 Heller R *et al.* *In vivo* gene electroinjection and expression in rat liver. *FEBS Lett* 1996; 389: 225-228.
- 30 Rois MP *et al.* *In vivo* electrically mediated protein and gene transfer in murine melanoma. *Nat Biotechnol* 1998; 16: 168-171.
- 31 Suzuki T *et al.* Direct gene transfer into rat liver cells by *in vivo* electroporation. *FEBS Lett* 1998; 425: 436-440.
- 32 Aihara H, Miyazaki J. Gene transfer into muscle by electroporation *in vivo*. *Nat Biotechnol* 1998; 16: 867-870.
- 33 Mir LM *et al.* Long-term, high level *in vivo* gene expression after electric pulse-mediated gene transfer into skeletal muscle. *C R Acad Sci III* 1998; 321: 893-899.
- 34 Lucas ML, Heller R. Immunomodulation by electrically enhanced delivery of plasmid DNA encoding IL-12 to murine skeletal muscle. *Mol Ther* 2001; 3: 47-53.
- 35 Mir LM *et al.* High-efficiency gene transfer into skeletal muscle mediated by electric pulses. *Proc Natl Acad Sci USA* 1999; 96: 4262-4267.
- 36 Gehl J, Mir LM. Determination of optimal parameters for *in vivo* gene transfer by electroporation, using a rapid *in vivo* test for cell permeabilization. *Biochem Biophys Res Commun* 1999; 261: 377-380.
- 37 Gehl J *et al.* *In vivo* electroporation of skeletal muscle: threshold, efficacy and relation to electric field distribution. *Biochim Biophys Acta* 1999; 1428: 233-240.
- 38 Bettan M *et al.* Efficient DNA electrotransfer into tumors. *Bioelectrochemistry* 2000; 52: 83-90.
- 39 Liu F, Huang L. Electric gene transfer to the liver following systemic administration of plasmid DNA. *Gene Therapy* 2002; 9: 1115-1119.
- 40 Lesbordes JC *et al.* *In vivo* electrotransfer of the cardiotrophin-1 gene into skeletal muscle slows down progression of motor neuron degeneration in pmn mice. *Hum Mol Genet* 2002; 11: 1615-1625.
- 41 Gehl J, Skovsgaard T, Mir LM. Vascular reactions to *in vivo* electroporation: characterization and consequences for drug and gene delivery. *Biochim Biophys Acta* 2002; 1569: 51-58.
- 42 Satkauskas S *et al.* Mechanisms of *in vivo* DNA electrotransfer: respective contributions of cell electroporation and DNA electrophoresis. *Mol Ther* 2002; 5: 133-140.
- 43 Bureau MF *et al.* Importance of association between permeabilization and electrophoretic forces for intramuscular DNA electrotransfer. *Biochim Biophys Acta* 2000; 1474: 353-359.
- 44 Puc M, Flisar K, Rebersek S, Mirkovic D. Electroporation for *in vitro* cell electroporation. *Radiol Oncol* 2001; 35: 203-207.
- 45 Zaharoff DA, Barr RC, Li CY, Yuan F. Electromobility of plasmid DNA in tumor tissues during electric field-mediated gene delivery. *Gene Therapy* 2002; 9: 1286-1290.
- 46 Lin CR *et al.* Electroporation-mediated pain-killer gene therapy for mononeuropathic rats. *Gene Therapy* 2002; 9: 1247-1253.
- 47 Lee TH *et al.* *In vivo* electroporation of proopiomelanocortin induces analgesia in a formalin-injection pain model in rats. *Pain* 2003; 104: 159-167.
- 48 Yu DS, Lee CE, Hsieh DS, Chang SY. Antitumor effects of recombinant BCG and interleukin-12 DNA vaccines on xenografted murine bladder cancer. *Urology* 2004; 63: 596-601.
- 49 Heller LC, Coppola D. Electrically mediated delivery of vector plasmid DNA elicits an antitumor effect. *Gene Therapy* 2002; 9: 1321-1325.
- 50 Kalat M *et al.* *In vivo* plasmid electroporation induces tumor antigen-specific CD8+ T-cell responses and delays tumor growth in a syngeneic mouse melanoma model. *Cancer Res* 2002; 62: 5489-5494.
- 51 Tamura T *et al.* Intratumoral delivery of interleukin 12 expression plasmids with *in vivo* electroporation is effective for colon and renal cancer. *Hum Gene Ther* 2001; 12: 1265-1276.
- 52 Tanaka M *et al.* Inhibition of RL male 1 tumor growth in BALB/c mice by introduction of the RL1 gene coding for antigen recognized by cytotoxic T-lymphocytes and the GM-CSF gene by *in vivo* electroporation. *Cancer Sci* 2004; 95: 154-159.

- 53 Yamashita YI *et al.* Electroporation-mediated interleukin-12 gene therapy for hepatocellular carcinoma in the mice model. *Cancer Res* 2001; 61: 1005-1012.
- 54 Lee CF, Chang SY, Hsieh DS, Yu DS. Treatment of bladder carcinomas using recombinant BCG DNA vaccines and electroporative gene immunotherapy. *Cancer Gene Ther* 2004; 11: 194-207.
- 55 Lee CF, Chang SY, Hsieh DS, Yu DS. Immunotherapy for bladder cancer using recombinant bacillus Calmette-Guerin DNA vaccines and interleukin-12 DNA vaccine. *J Urol* 2004; 171: 1343-1347.
- 56 Lucas ML, Heller L, Coppola D, Heller R. IL-12 plasmid delivery by *in vivo* electroporation for the successful treatment of established subcutaneous B16.F10 melanoma. *Mol Ther* 2002; 5: 668-675.
- 57 Matsubara H *et al.* Electroporation-mediated transfer of cytokine genes into human esophageal tumors produces anti-tumor effects in mice. *Anticancer Res* 2001; 21: 2501-2503.
- 58 Heller R *et al.* Intradermal delivery of interleukin-12 plasmid DNA by *in vivo* electroporation. *DNA Cell Biol* 2001; 20: 21-26.
- 59 Heller L *et al.* *In vivo* electroporation of plasmids encoding GM-CSF or interleukin-2 into existing B16 melanomas combined with electrochemotherapy induces long-term antitumor immunity. *Melanoma Res* 2000; 10: 577-583.
- 60 Kishida T *et al.* *In vivo* electroporation-mediated transfer of interleukin-12 and interleukin-18 genes induces significant antitumor effects against melanoma in mice. *Gene Therapy* 2001; 8: 1234-1240.
- 61 Kishida T *et al.* Electrochemo-gene therapy of cancer: intratumoral delivery of interleukin-12 gene and bleomycin synergistically induced therapeutic immunity and suppressed subcutaneous and metastatic melanomas in mice. *Mol Ther* 2003; 8: 738-745.
- 62 Li S *et al.* Intramuscular electroporation delivery of IFN- α gene therapy for inhibition of tumor growth located at a distant site. *Gene Therapy* 2001; 8: 400-407.
- 63 Li S, Zhang X, Xia X. Regression of tumor growth and induction of long-term antitumor memory by interleukin 12 electro-gene therapy. *J Natl Cancer Inst* 2002; 94: 762-768.
- 64 Li S, Xia X, Zhang X, Suen J. Regression of tumors by IFN- α electroporation gene therapy and analysis of the responsible genes by cDNA array. *Gene Therapy* 2002; 9: 390-397.
- 65 Zhang GH *et al.* Gene expression and antitumor effect following *in vivo* electroporation delivery of human interferon alpha 2 gene. *Acta Pharmacol Sin* 2003; 24: 891-896.
- 66 Lucas ML, Heller R. IL-12 gene therapy using an electrically mediated nonviral approach reduces metastatic growth of melanoma. *DNA Cell Biol* 2003; 22: 755-763.
- 67 Tamura T *et al.* Combination of IL-12 and IL-18 of electro-gene therapy synergistically inhibits tumor growth. *Anticancer Res* 2003; 23: 1173-1179.
- 68 Chi CH, Wang YS, Lai YS, Chi KH. Anti-tumor effect of *in vitro* IL-2 and GM-CSF electrogene therapy in murine hepatoma model. *Anticancer Res* 2003; 23: 315-321.
- 69 Heller LC *et al.* Effect of electrically mediated intratumor and intramuscular delivery of a plasmid encoding IFN alpha on visible B16 mouse melanomas. *Technol Cancer Res Treat* 2002; 1: 205-209.
- 70 Lee SC *et al.* Inhibition of established subcutaneous and metastatic murine tumors by intramuscular electroporation of the interleukin-12 gene. *J Biomed Sci* 2003; 10: 73-86.
- 71 Baba M, Iishi H, Tatsuta M. Transfer of bcl-xS plasmid is effective in preventing and inhibiting rat hepatocellular carcinoma induced by N-nitrosomorpholine. *Gene Therapy* 2001; 8: 1149-1156.
- 72 Jiang Y *et al.* Stimulation of mammary tumorigenesis by systemic tissue inhibitor of matrix metalloproteinase 4 gene delivery. *Cancer Res* 2001; 61: 2365-2370.
- 73 Goto T *et al.* Highly efficient electro-gene therapy of solid tumor by using an expression plasmid for the herpes simplex virus thymidine kinase gene. *Proc Natl Acad Sci USA* 2000; 97: 354-359.
- 74 Mikata K *et al.* Inhibition of growth of human prostate cancer xenograft by transfection of p53 gene: gene transfer by electroporation. *Mol Cancer Ther* 2002; 1: 247-252.
- 75 Shibata MA, Morimoto J, Otsuki Y. Suppression of murine mammary carcinoma growth and metastasis by HSVtk/GCV gene therapy using *in vivo* electroporation. *Cancer Gene Ther* 2002; 9: 16-27.
- 76 Hsieh YH *et al.* Electroporation-mediated and EBV LMP1-regulated gene therapy in a syngenic mouse tumor model. *Cancer Gene Ther* 2003; 10: 626-636.
- 77 Cemazar M *et al.* Effects of electrogenotherapy with p53wt combined with cisplatin on survival of human tumor cell lines with different p53 status. *DNA Cell Biol* 2003; 22: 765-775.
- 78 Ivanov MA *et al.* Enhanced antitumor activity of a combination of MBD2-antisense electrotransfer gene therapy and bleomycin electrochemotherapy. *J Gene Med* 2003; 5: 893-899.
- 79 Slack A *et al.* Antisense MBD2 gene therapy inhibits tumorigenesis. *J Gene Med* 2002; 4: 381-389.
- 80 Shibata MA, Horiguchi T, Morimoto J, Otsuki Y. Massive apoptotic cell death in chemically induced rat urinary bladder carcinomas following *in situ* HSVtk electrogene transfer. *J Gene Med* 2003; 5: 219-231.
- 81 Wang F *et al.* Inhibition of tumor angiogenesis, growth and metastasis by blocking VEGF paracrine pathway. *Sheng Wu Hua Xue Yu Sheng Wu Wu Li Xue Bao (Shanghai)* 2002; 34: 165-170.
- 82 Yoshizato K *et al.* Gene delivery with optimized electroporation parameters shows potential for treatment of gliomas. *Int J Oncol* 2000; 16: 899-905.
- 83 Trochon-Joseph V *et al.* Evidence of antiangiogenic and antimetastatic activities of the recombinant disintegrin domain of metargidin. *Cancer Res* 2004; 64: 2062-2069.
- 84 Niu G *et al.* Gene therapy with dominant-negative Stat3 suppresses growth of the murine melanoma B16 tumor *in vivo*. *Cancer Res* 1999; 59: 5059-5063.
- 85 Martel-Renoir D *et al.* Coelectrotransfer to skeletal muscle of three plasmids coding for antiangiogenic factors and regulatory factors of the tetracycline-inducible system: tightly regulated expression, inhibition of transplanted tumor growth, and antimetastatic effect. *Mol Ther* 2003; 8: 425-433.
- 86 Cichon T *et al.* Electrotransfer of gene encoding endostatin into normal and neoplastic mouse tissues: inhibition of primary tumor growth and metastatic spread. *Cancer Gene Ther* 2002; 9: 771-777.
- 87 Matsubara H *et al.* Combinatory anti-tumor effects of electroporation-mediated chemotherapy and wild-type p53 gene transfer to human esophageal cancer cells. *Int J Oncol* 2001; 18: 825-829.
- 88 Mallat Z *et al.* Interleukin-18/interleukin-18 binding protein signaling modulates atherosclerotic lesion development and stability. *Circ Res* 2001; 89: E41-E45.
- 89 Hase M, Tanaka M, Yokota M, Yamada Y. Reduction in the extent of atherosclerosis in apolipoprotein E-deficient mice induced by electroporation-mediated transfer of the human plasma platelet-activating factor acetylhydrolase gene into skeletal muscle. *Prostaglandins Other Lipid Mediators* 2002; 70: 107-118.
- 90 Silvestre JS *et al.* Antiangiogenic effect of interleukin-10 in ischemia-induced angiogenesis in mice hindlimb. *Circ Res* 2000; 87: 448-452.
- 91 Tanaka S, Uehara T, Nomura Y. Up-regulation of protein-disulfide isomerase in response to hypoxia/brain ischemia and its protective effect against apoptotic cell death. *J Biol Chem* 2000; 275: 10388-10393.

- 92 Mallat Z *et al.* Interleukin-18/interleukin-18 binding protein signaling modulates ischemia-induced neovascularization in mice hindlimb. *Circ Res* 2002; 91: 441-448.
- 93 Silvestre JS *et al.* Vascular endothelial growth factor-B promotes *in vivo* angiogenesis. *Circ Res* 2003; 93: 114-123.
- 94 Adachi O *et al.* Gene transfer of Fc-fusion cytokine by *in vivo* electroporation: application to gene therapy for viral myocarditis. *Gene Therapy* 2002; 9: 577-583.
- 95 Deleuze V, Scherman D, Bureau MF. Interleukin-10 expression after intramuscular DNA electrotransfer: kinetic studies. *Biochem Biophys Res Commun* 2002; 299: 29-34.
- 96 Nakano A *et al.* Cytokine gene therapy for myocarditis by *in vivo* electroporation. *Hum Gene Ther* 2001; 12: 1289-1297.
- 97 Watanabe K *et al.* Protection against autoimmune myocarditis by gene transfer of interleukin-10 by electroporation. *Circulation* 2001; 104: 1098-1100.
- 98 Babluc S *et al.* Electroporation improves the efficacy of DNA vaccines in large animals. *Vaccine* 2002; 20: 3399-3408.
- 99 Bachy M *et al.* Electric pulses increase the immunogenicity of an influenza DNA vaccine injected intramuscularly in the mouse. *Vaccine* 2001; 19: 1688-1693.
- 100 Nomura M *et al.* *In vivo* induction of cytotoxic T lymphocytes specific for a single epitope introduced into an unrelated molecule. *J Immunol Methods* 1996; 193: 41-49.
- 101 Paster W *et al.* *In vivo* plasmid DNA electroporation generates exceptionally high levels of epitope-specific CD8+ T-cell responses. *Gene Therapy* 2003; 10: 717-724.
- 102 Peretz Y, Zhou ZF, Halwani E, Prud'homme GJ. *In vivo* generation of dendritic cells by intramuscular codelivery of FLT3 ligand and GM-CSF plasmids. *Mol Ther* 2002; 6: 407-414.
- 103 Selby M *et al.* Enhancement of DNA vaccine potency by electroporation *in vivo*. *J Biotechnol* 2000; 83: 147-152.
- 104 Tjelle TE *et al.* Monoclonal antibodies produced by muscle after plasmid injection and electroporation. *Mol Ther* 2004; 9: 328-336.
- 105 Tøllersen S *et al.* DNA injection in combination with electroporation: a novel method for vaccination of farmed ruminants. *Scand J Immunol* 2003; 57: 229-238.
- 106 Widera G *et al.* Increased DNA vaccine delivery and immunogenicity by electroporation *in vivo*. *J Immunol* 2000; 164: 4635-4640.
- 107 Zucchielli S *et al.* Enhancing B- and T-cell immune response to a hepatitis C virus E2 DNA vaccine by intramuscular electrical gene transfer. *J Virol* 2000; 74: 11598-11607.
- 108 Yang L *et al.* Generation of monoclonal antibodies against Blo 13 using DNA immunization with *in vivo* electroporation. *Clin Exp Allergy* 2003; 33: 663-668.
- 109 Wu CJ, Lee SC, Huang HW, Tao MH. *In vivo* electroporation of skeletal muscles increases the efficacy of Japanese encephalitis virus DNA vaccine. *Vaccine* 2004; 22: 1457-1464.
- 110 Aurisicchio L *et al.* Tamarin alpha-interferon is active in mouse liver upon intramuscular gene delivery. *J Gene Med* 2001; 3: 394-402.
- 111 Perez N *et al.* Regulatable systemic production of monoclonal antibodies by *in vivo* muscle electroporation. *Genet Vaccines Ther* 2004; 2: 2.
- 112 Deleuze V *et al.* LPS-induced bronchial hyperreactivity: interference by mIL-10 differs according to site of delivery. *Am J Physiol Lung Cell Mol Physiol* 2004; 286: L98-L105.
- 113 Maruyama H *et al.* Long-term production of erythropoietin after electroporation-mediated transfer of plasmid DNA into the muscles of normal and uremic rats. *Gene Therapy* 2001; 8: 461-468.
- 114 Maruyama H *et al.* Skin-targeted gene transfer using *in vivo* electroporation. *Gene Therapy* 2001; 8: 1808-1812.
- 115 Nordstrom JL. The antiprogesterone-dependent GeneSwitch system for regulated gene therapy. *Steroids* 2003; 68: 1085-1094.
- 116 Ataka K *et al.* Effects of erythropoietin-gene electrotransfer in rats with adenine-induced renal failure. *Am J Nephrol* 2003; 23: 315-323.
- 117 Kreiss P, Bettan M, Crouzet J, Scherman D. Erythropoietin secretion and physiological effect in mouse after intramuscular plasmid DNA electrotransfer. *J Gene Med* 1999; 1: 245-250.
- 118 Rizzuto G *et al.* Gene electrotransfer results in a high-level transduction of rat skeletal muscle and corrects anemia of renal failure. *Hum Gene Ther* 2000; 11: 1891-1900.
- 119 Terada Y *et al.* Efficient and ligand-dependent regulated erythropoietin production by naked DNA injection and *in vivo* electroporation. *Am J Kidney Dis* 2001; 38: S50-S53.
- 120 Rizzuto G *et al.* Efficient and regulated erythropoietin production by naked DNA injection and muscle electroporation. *Proc Natl Acad Sci USA* 1999; 96: 6417-6422.
- 121 Dalle B *et al.* Dimeric erythropoietin fusion protein with enhanced erythropoietic activity *in vitro* and *in vivo*. *Blood* 2001; 97: 3776-3782.
- 122 Lamartina S *et al.* Stringent control of gene expression *in vivo* by using novel doxycycline-dependent trans-activators. *Hum Gene Ther* 2002; 13: 199-210.
- 123 Horiki M *et al.* High-level expression of interleukin-4 following electroporation-mediated gene transfer accelerates Type 1 diabetes in NOD mice. *J Autoimmun* 2003; 20: 111-117.
- 124 Martinenghi S *et al.* Human insulin production and amelioration of diabetes in mice by electrotransfer-enhanced plasmid DNA gene transfer to the skeletal muscle. *Gene Therapy* 2002; 9: 1429-1437.
- 125 Prud'homme GJ, Chang Y, Li X. Immunoinhibitory DNA vaccine protects against autoimmune diabetes through cDNA encoding a selective CTLA-4 (CD152) ligand. *Hum Gene Ther* 2002; 13: 395-406.
- 126 Yin D, Tang JG. Gene therapy for streptozotocin-induced diabetic mice by electroporation transfer of naked human insulin precursor DNA into skeletal muscle *in vivo*. *FEBS Lett* 2001; 495: 16-20.
- 127 Wang LY, Sun W, Chen MZ, Wang X. Intramuscular injection of naked plasmid DNA encoding human preproinsulin gene in streptozotocin-diabetic mice results in a significant reduction of blood glucose level. *Sheng Li Xue Bao* 2003; 55: 641-647.
- 128 Rabinovsky ED, Draghia-Akli R. Insulin-like growth factor I plasmid therapy promotes *in vivo* angiogenesis. *Mol Ther* 2004; 9: 46-55.
- 129 Pradat PF *et al.* Partial prevention of cisplatin-induced neuropathy by electroporation-mediated nonviral gene transfer. *Hum Gene Ther* 2001; 12: 367-375.
- 130 Zang WP *et al.* Transfer and expression of recombinant human thrombopoietin gene in COS-7 Cells and mice *in vivo*. *Zhongguo Shi Yan Xue Ye Xue Za Zhi* 2001; 9: 14-17.
- 131 Zang WP, Wei XD, Wang SW, Wang DB. Thrombopoietic effect of recombinant human thrombopoietin gene transferred to mice mediated by electric pulse on normal and experimental thrombocytopenia mice. *Zhonghua Xue Ye Xue Za Zhi* 2001; 22: 126-131.
- 132 Payen E *et al.* Improvement of mouse beta-thalassemia by electrotransfer of erythropoietin cDNA. *Exp Hematol* 2001; 29: 295-300.
- 133 Samakoglu S *et al.* beta-Minor-globin messenger RNA accumulation in reticulocytes governs improved erythropoiesis in beta thalassemic mice after erythropoietin complementary DNA electrotransfer in muscles. *Blood* 2001; 97: 2213-2220.
- 134 Fewell JG *et al.* Gene therapy for the treatment of hemophilia B using PNC-formulated plasmid delivered to muscle with electroporation. *Mol Ther* 2001; 3: 574-583.
- 135 Tomanin R *et al.* Non-viral transfer approaches for the gene therapy of mucopolysaccharidosis type II (Hunter syndrome). *Acta Paediatr Suppl* 2002; 91: 100-104.
- 136 Gollins H, McMahon J, Wells KE, Wells DJ. High-efficiency plasmid gene transfer into dystrophic muscle. *Gene Therapy* 2003; 10: 504-512.

- 137 Vilquin JT *et al.* Electrotransfer of naked DNA in the skeletal muscles of animal models of muscular dystrophies. *Gene Therapy* 2001; 8: 1097-1107.
- 138 Murakami T *et al.* Full-length dystrophin cDNA transfer into skeletal muscle of adult mdx mice by electroporation. *Muscle Nerve* 2003; 27: 237-241.
- 139 Brigueot A *et al.* Transcriptional activation of the utrophin promoter B by a constitutively active Ets-transcription factor. *Neuromuscul Disord* 2003; 13: 143-150.
- 140 Ferrer A *et al.* Long-term expression of full-length human dystrophin in transgenic mdx mice expressing internally deleted human dystrophins. *Gene Therapy* 2004; 11: 884-893.
- 141 Chuang IC *et al.* Intramuscular electroporation with the pro-opiomelanocortin gene in rat adjuvant arthritis. *Arthritis Res Ther* 2004; 6: R7-R14.
- 142 Kim JM *et al.* Electro-gene therapy of collagen-induced arthritis by using an expression plasmid for the soluble p75 tumor necrosis factor receptor-Fc fusion protein. *Gene Therapy* 2003; 10: 1216-1224.
- 143 Perez N *et al.* Tetracycline transcriptional silencer tightly controls transgene expression after *in vivo* intramuscular electrotransfer: application to interleukin 10 therapy in experimental arthritis. *Hum Gene Ther* 2002; 13: 2161-2172.
- 144 Saidenberg-Kermanach N *et al.* Efficacy of interleukin-10 gene electrotransfer into skeletal muscle in mice with collagen-induced arthritis. *J Gene Med* 2003; 5: 164-171.
- 145 Bloquel C, Fabre E, Bureau MF, Scherman D. Plasmid DNA electrotransfer for intracellular and secreted proteins expression: new methodological developments and applications. *J Gene Med* 2004; 6 (Suppl 1): S11-S23.
- 146 Kishimoto KN, Watanabe Y, Nakamura H, Kokubun S. Ectopic bone formation by electroporative transfer of bone morphogenetic protein-4 gene. *Bone* 2002; 31: 340-347.
- 147 Magee TK *et al.* Gene therapy of erectile dysfunction in the rat with penile neuronal nitric oxide synthase. *Biol Reprod* 2002; 67: 1033-1041.
- 148 Yasui A *et al.* Elevated gastrin secretion by *in vivo* gene electroporation in skeletal muscle. *Int J Mol Med* 2001; 8: 489-494.
- 149 Tanaka T *et al.* *In vivo* gene transfer of hepatocyte growth factor to skeletal muscle prevents changes in rat kidneys after 5/6 nephrectomy. *Am J Transplant* 2002; 2: 828-836.
- 150 Xue F *et al.* Attenuated acute liver injury in mice by naked hepatocyte growth factor gene transfer into skeletal muscle with electroporation. *Gut* 2002; 50: 558-562.
- 151 Riera M *et al.* Intramuscular SP1017-formulated DNA electrotransfer enhances transgene expression and distributes hHGF to different rat tissues. *J Gene Med* 2004; 6: 111-118.
- 152 Takahashi T *et al.* IGF-I gene transfer by electroporation promotes regeneration in a muscle injury model. *Gene Therapy* 2003; 10: 612-620.
- 153 Sakamoto T *et al.* Target gene transfer of tissue plasminogen activator to cornea by electric pulse inhibits intracameral fibrin formation and corneal cloudiness. *Hum Gene Ther* 1999; 10: 2551-2557.
- 154 Yomogida K, Yagura Y, Nishimune Y. Electroporated transgene-rescued spermatogenesis in infertile mutant mice with a sertoli cell defect. *Biol Reprod* 2002; 67: 712-717.
- 155 Ochiai H *et al.* Synthesis of human erythropoietin *in vivo* in the oviduct of laying hens by localized *in vivo* gene transfer using electroporation. *Poult Sci* 1998; 77: 299-302.
- 156 Draghia-Akli R *et al.* High-efficiency growth hormone-releasing hormone plasmid vector administration into skeletal muscle mediated by electroporation in pigs. *FASEB J* 2003; 17: 526-528.
- 157 Khan AS *et al.* Maternal GHRH plasmid administration changes pituitary cell lineage and improves progeny growth of pigs. *Am J Physiol Endocrinol Metab* 2003; 285: E224-E231.
- 158 Draghia-Akli R *et al.* Effects of plasmid-mediated growth hormone releasing hormone supplementation in young, healthy Beagle dogs. *J Anim Sci* 2003; 81: 2301-2310.
- 159 <http://www.cliniporator.com>.